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OBSERVATIONS ON MENINGOCOCCUS CARRIERS AND ON THE BACTERIOLOGY OF EPIDEMIC MENINGITIS

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Because epidemic meningitis is especially prone to occur in military camps, the problems relating to the control of this disease are of particular interest at this time. The exact conditions under which this disease becomes epidemic have not been determined. It seems probable that the number of susceptible individuals in any community is very small and that crowded living quarters, fatigue, exposure, and frequent changes in the population are factors which favor the development of the infection. It is the accepted view that the disease is spread largely by direct contact with healthy persons harboring meningococci in the nose and throat rather than by patients. Accordingly, at the present time, the most important procedure relied on for the control of epidemic meningitis involves the identification and detention of healthy meningococcus carriers. The weight of experience so far at hand favors this procedure, but more evidence is needed to determine its exact value. Such evidence must necessarily come from painstaking bacteriologic investigations of epidemics of meningitis. Recently we have been engaged in the study of this disease in a large military camp. In this work bacteriologic examinations were made of more than 15,000 men, and a number of meningococcus carriers were identified. In an extended study of these carriers certain observations have been made which are of sufficient interest to be recorded. Furthermore, a large number of strains of meningococci have been obtained, both from the nasopharynx of carriers, and from the spinal fluid of infected individuals in different localities. A careful comparative study of the cultural and biologic characters of these organisms have been carried out and the results are included in this article.

Received for publication Jan. 2, 1918.

BACTERIOLOGIC TECHNIC WITH SPECIAL REFERENCE TO CARRIER DETECTION

At the beginning of this work it was necessary to develop a technic for the identification of the meningococcus which was simple, reliable, and practical. In our hands the technic herein described fulfils these requirements.

Since in the carrier the meningococcus most frequently inhabits the secretions of the posterior nares and nasopharynx, cultures must be made from these sources. In our experience the necessary material is obtained best by means of a simple swab made by wrapping cotton about the end of a piece of stovepipe wire about 25 cm. long. The straight swabs are sterilized in the autoclave in test tubes and then bent near the ends over the edge of the tube to an angle of 45 degrees as each is withdrawn for use. To make the smear the tongue is depressed, and during phonation the swab is passed behind the soft palate and a thorough smear of the posterior nasopharyngeal surfaces made; as the swab is removed care is taken not to touch its posterior surface against the throat or tongue. A small portion of material on the posterior surface of the swab is immediately placed on the surface of the plated culture medium, near the edge of the plate, and streaks are made from this material in various directions with a sterile wire needle. When possible only one culture should be made on a plate and the plate should be placed in the incubator without delay; a few hours at ordinary summer temperature however does not seem to affect the results appreciably, but in colder weather the plates must be warmed to 37 C. and packed in suitable containers if they are to be transported even comparatively short distances without danger. The plate cultures previously warmed to 37 C. should not be kept out of the incubator longer than 1 hour after inoculation. The swabs are flamed or dipped in a strong disinfectant after using.

Careful comparison of a large number of culture mediums recommended for this work has been made, and we find that plain blood agar gives the most satisfactory results. It is a medium that is easily prepared and at present everywhere easily obtained in this country. Bottles or flasks with 100-500 c c of plain agar are handy for this work. Defibrinated blood, either human, horse, goat or sheep, is easily procured and small amounts suffice for a large number of plates. After melting the agar in the flask or bottle it is cooled down to 45-47 C. when blood is added in a proportion of 5-10%. It is well to warm the blood to about 40 C. before adding it to the agar. After mixing, the blood agar is poured into sterile petri dishes and allowed to cool. By incubation, tests for sterility of the medium are made.

The meningococcus is a gram-negative diplococcus. Other members of the group of gram-negative diplococci, except the gonococcus, frequently inhabit the posterior nasopharynx and the differentiation of the meningococcus from these other gram-negative diplococci is a most important step in carrier detection. On blood agar the meningococcus colonies vary in size, are very moist, semitranslucent, elevated, sticky in consistency, and of indistinct outline; often colonies coalesce in irregular masses. When viewed in transmitted light the colonies have a faint bluish gray tint and are almost transparent. Micrococcus catarrhalis cannot be differentiated from the meningococcus by the appearance of the colonies in blood-agar plate cultures, although the catarrhalis colonies are often larger and more opaque than the meningococcus colonies. According to our experience growth at room temperature or the use of transparent culture medium has little practical value in the differentiation of the meningococcus and Micrococcus catarrhalis. Here we would state that the use of blood agar has a great advantage over transparent mediums in that the members of the streptococcus-pneumococcus group are easily differentiated from the meningococcus by the appearance of the colonies. This is an important point for these organisms are often found in cultures and the colonies resemble meningococcus colonies on transparent mediums. In the instance of Micrococcus pharyngis siccus and Micrococcus flavus the characteristics of the colonies are such that the 24-hour culture on blood-agar plates usually suffices for differentiation from the meningococcus. Micrococcus pharyngis siccus colonies are medium sized, flat, grayish in color, dry, and often wrinkled on the surface. The flavus group of organisms in young cultures resemble the meningococcus in the appearance of their colonies. After 24-36 hours' incubation, however, the colonies become yellowish in color, opaque, somewhat dry, and pointlike in consistency. Colonies of the flavus group on blood-agar plates can usually be identified by these characteristics, but in some instances agglutination tests are necessary for the final diagnosis of the organism.

The blood-agar plate cultures of the nasopharyngeal material are examined after 16-24 hours' incubation and the suspicious colonies transferred to blood-agar slants. It is important that all tubes used in meningococcus cultures be warmed to 37 C. before inoculation. The subcultures are incubated for 8-16 hours at 37 C. and used for identification tests. In making nasopharyngeal cultures time can be saved by taking the swabs in the late afternoon, incubating the cultures overnight; transfers made from the suspicious colonies on the following morning, grow out sufficiently for agglutination tests the same afternoon. This procedure makes it possible to report the final results of cultures in 36 hours. The original plates are set aside at room temperature and the suspicious colonies again examined 12-24 hours later for the development of pigment.

For agglutination tests polyvalent antimeningococcus serum of high potency is used in dilutions of 1/50, 1/100, 1/500; also normal horse serum in a dilution of 1/50 for control. Equal parts of the serum and a suspension of the bacteria in salt solution (the growth from an ordinary 24-hour blood-agar slant culture of meningococci in 3 or 4 c c of normal salt solution) are mixed and incubated for 6-12 hours at a temperature of 55 C. If suspensions of a given coccus are agglutinated in high dilutions with the antimeningococcic serum, and not by the normal serum, the organism is treated as a meningococcus, and the person from whom the culture was obtained is isolated as a carrier. The microscopic test devised by Tunnicliff[1] is also reliable and adapted to more rapid diagnosis.

A satisfactory medium for fermentative tests is the Hiss serum water containing litmus as an indicator and 1% of the test sugar. The fermentative reactions of the gram-negative diplococci concerned are such that glucose and saccharose are the only sugar necessary as a rule. The meningococcus ferments glucose and not saccharose; Micrococcus catarrhalis does not change either sugar; while the other members of this group occurring in the nasopharynx change both glucose and saccharose. As a measure of economy the sugar mediums are put up in amounts of 2 c c in small test tubes 1 cm. in diameter. Transfers are usually made to the sugar mediums from the first subcultures from the suspicious colonies. The sugar medium cultures are tested for growth after

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[1] Jour. Am. Med. Assn., 1917, 69, 786.

24 hours' incubation. These cultures however must be incubated 5-7 days before final readings, and great care must be exercised to avoid contamination. The fermentative reactions coincide with the results of the agglutination tests as a rule.

In making cultures of cerebrospinal fluid the sediment of a centrifugalized specimen of freshly drawn fluid is planted on blood-agar slants as well as in tubes of 1% dextrose broth containing serum or ascites fluid. In the instances in which the spinal fluid does not yield a sediment on centrifugalization the whole fluid is planted in 1% dextrose broth containing serum or ascites fluid. The broth cultures of spinal fluid should be incubated from 3-5 days before they are discarded. Using these methods meningococci were isolated from the spinal fluids of meningitis patients in practically all instances. The cultural characteristics of each organism as well as the biologic characters are determined in each instance. The stock cultures are kept on defibrinated blood agar at room temperature. Transfers are made every 24-48 hours to insure the life of the culture.

RESULTS

As soon as a case of meningitis occurred the immediate contacts were examined bacteriologically, the carriers identified and isolated. If more than one case occurred in an organization, the entire organization was examined for carriers. It was found that 3-6% of the men examined harbored meningococci in the secretions of the nose and throat. The number of carriers in different groups of men varied (Table 1). One hundred and forty immediate contacts (tentmates of cases of meningitis) were examined, in most instances within 12 hours after the case of meningitis was found; 26.7% of these were found to be meningococcus carriers. Seven of the immediate contacts, or 5%, proved to be chronic carriers. Thirty-eight patients, convalescent from the disease, were cultured and 38.2% were identified as carriers. All were discharged, however, in a few weeks with negative cultures. At the time of the epidemic 13.5% of the hospital attendants yielded positive meningococcus cultures. In this group the

TABLE 1

SUMMARY OF EXAMINATIONS FOR MENINGOCOCCUS CARRIERS

Source of Material Examined	Number Examined	Percentage
Convalescents from epidemic meningitis	38	38.2
Hospital corps	46	13.5
Immediate contacts	140	26.7
Other members of organizations in which epidemic meningitis occurred	15,257	4.4
Total number of persons examined 1.2% were found to be chronic carriers	15,351	

nurses taking care of the meningitis cases were included. Of other men, members of organizations in which cases of meningitis had occurred, 15,257 in all, 4.4% yielded positive meningococcus cultures. Of all the persons examined 1.2% proved to be chronic carriers.

It seems important at this point to emphasize the distinction between two tyes of meningococcus carriers. The chronic carriers, individuals yielding positive nasopharyngeal cultures over long periods of time, were few in number and only in rare instances developed meningitis. In the cultures from these persistent carriers the meningococci in most cases were present in predominating numbers; indeed many pure cultures were obtained in such cases. In our experience the connection between chronic meningococcus carriers and cases of meningitis has been striking in many instances, and seem to us to indicate the necessity of special attention being given to this type of carrier in all epidemics of this disease. On the other hand the temporary carriers were more numerous and in the cultures from these individuals the number of meningococci was small as a rule. These persons seem to harbor meningococci in their nasopharynx for a short period of time only.

During the course of this work repeated bacteriologic examinations have been made of the interns, nurses, and attendants coming in contact with cases of epidemic meningitis in the hospital of the Memorial Institute, and no meningococcus carriers have been found. It seems reasonable to regard this result as due in large degree to the use of the precautionary measures, including the wearing of face-masks, described by Weaver.[2]

A point or two in regard to the bacteriologic examination of meningococcus carriers may be emphasized here. The intricacy of the bacteriologic methods involved and the anatomy of the nasopharyngeal structures make the detection of the meningococcus carrier a rather difficult procedure. A certain small number of carriers especially those persons who have only a few menigococci in the nasopharyngeal secretions no doubt are overlooked. Furthermore one or two negative cultures may be obtained from chronic carriers who yield positive cultures on subsequent examination. The taking of the swab should be as thorough as possible for the meningococcus seems to grow in isolated foci in the upper respiratory tract.[2a] Before a chronic carrier is discharged from isolation it seems advisable to obtain at least 3 successive negative cultures. Perhaps these persons should be under close observation for a time even after they return to their organizations.

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[2] Jour. Am. Med. Assn., 1918, 70, p. 76.

[2a] Herrold, R. D.: Jour. Am. Med. Assn., 1918, 70, p. 82.

BIOLOGIC REACTIONS OF MENINGOCOCCI FROM DIFFERENT SOURCES

The classification of meningococci on the basis of biologic differences was first attempted by Dopter[3] in 1909. Dopter isolated organisms from the nasopharynx of contacts which resembled meningococci in every characteristic except serum reactions. He also, a little later, demonstrated these atypical organisms in the spinal fluid of cases of meningitis, and on the basis of biologic differences he established two main groups—meningococcus and parameningococcus. Dopter's work has been confirmed and extended by many investigators, particularly Wollstein[4] in America, Gordon,[5] Arkwright,[6] Ellis,[7] Fildes and Baker,[8] and others in England, Nicolle,[9] and others in France. From these observations it appears that the meningococci fall into two large biologic groups: The typical meningococcus (Group 1) and the parameningococcus (Group 2). A great variety of intermediate strains related to one or the other of the two large groups occur, however, and Gordon has classified these intermediates in 2 groups which he calls Groups 3 and 4. Biologic differences in meningococcus strains are of great practical importance in the identification of the organism and in the preparation of good therapeutic serum. Moreover, problems relating to the meningococcus carrier can be studied more efficiently on the basis of such differentiation.

By the use of biologic reactions we have been able to compare meningococci obtained from cases of meningitis and from carriers in the same and different localities with interesting results.

In this phase of the work monovalent serum of high potency was prepared for known pathogenic strains of meningococci, that is, organisms isolated from the spinal fluids in cases of meningitis. In the preparation of serum young rabbits weighing 1,500-2,000 gm. were used, and a monovalent serum having an agglutination titer of 100-300 or higher for its homologous organism was considered satisfactory for the experiments.

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[3] Compt. rend. Soc. de biol., 1909, 67, p. 74.

[4] Jour. Exper. Med., 1914, 20, p. 201.

[5] Jour. Royal Army Med. Corps, 1915, 25, p. 411. Also Medical Research Committee, Special Report, Series No. 3, 1917.

[6] Brit. Med. Jour., 1915, (2) p. 885.

[7] Ibid., p. 881.

[8] Lancet, 1918, 194, p. 92.

[9] Bull. et mém. de la Soc. méd. d. Hôp., 1917, 41, p. 878.

In the agglutination tests unheated monovalent serum was used in dilutions of 1/50, 1/100, 1/200, 1/300, 1/400, 1/500, 1/600, 1/700, 1/800, 1/900 and 1/1000, and normal rabbit serum in dilutions of 1/50 and 1/100 for controls. The bacterial suspensions were prepared by suspending the growth from one 24-hour blood-agar slant culture of the organism to be tested in 4 c c of sterile normal salt solution. By this means moderately turbid suspensions were obtained. Equal amounts, usually 0.25 c c, of bacterial emulsion and the different dilutions of immune and normal serum were mixed, and incubated for 24 hours at 55 C. A positive agglutination was recorded only in those instances in which agglutination was complete. In all the agglutination tests the macroscopic method was used.

TABLE 2

Biologic Reactions of Meningococci Obtained from the Spinal Fluid of Cases of Meningitis

Strain of Meningococcus	Serum											
	2406	2425	2379	2385	2363	2457	2353	2372	A	B	C	D
2406	400	800	700	100	400	1000	500	200	200	0	300	0
2425	800	900	600	200	500	400	500	600	400	0	800	0
2379	1000	1000	500	100	300	1000	1000	300	1000	0	500	0
2385	1000	200	1000	400	1000	1000	900	1000	500	500	500	200
2363	300	500	500	200	900	600	300	300	300	0	400	0
2457	1000	1000	700	50	800	300	700	500	200	0	500	0
2353	1000	1000	800	100	500	500	600	400	100	0	300	0
2372	800	1000	800	300	800	400	700	300	500	0	400	0
A	1000	1000	700	200	500	1000	1000	400	500	0	300	0
B	0	0	0	0	0	0	0	0	0	500	100	600
C	700	1000	900	200	1000	1000	400	800	800	100	1000	100
D	0	0	0	0	0	0	0	0	0	0	0	10?

Using these methods 36 strains of meningococci from the cerebrospinal fluid of cases of meningitis and 114 strains from the nasopharyngeal secretions of chronic carriers have been examined. All of these organisms had the morphologic and cultural characters of the meningococcus and were in most instances agglutinated by a polyvalent antimeningococcus serum. In a series of cross agglutinations using 12 of the pathogenic strains of meningococci from sporadic cases of meningitis occurring in Chicago, and their homologous immune serums, 2 biologic groups were demonstrated. In one group there were 2 and in the other group 10 strains.

The results of these tests are recorded in Table 2. In the initial experiments the agglutination titer of the monovalent serums for the heterologous strains varied, and in a few instances cross agglutination between the two groups occurred. These variations in agglutinability, however, did not seem of sufficient degree to warrant more complex biologic division. One of the groups corresponds to the typical meningococcus, and the other to the parameningococcus, according to cross agglutination tests using strains of these organisms obtained from the Laboratory of the United States Public Health Service, Washington, D. C.

The biologic characters of strains of meningococci from cases of epidemic meningitis and chronic carriers occurring in Chicago, and in the armed camp during the epidemic, as determined by agglutination tests with representative serum of high titer for each of these biologic groups have been studied (Table 3).

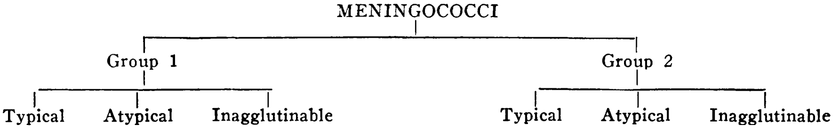
TABLE 3
BIOLOGIC CLASSIFICATION OF MENINGOCOCCI FROM DIFFERENT SOURCES AS DETERMINED BY AGGLUTINATION

Source of Strains	Group 1		Group 2	
	No. of Strains	Per cent. of Total	No. of Strains	Per cent. of Total
Cerebrospinal fluid, meningitis. Military camp	12	85.7	2	14.3
Nasopharynx of carriers. Military camp	95	83.3	19	16.7
Cerebrospinal fluid, meningitis. City of Chicago	3	13.7	19	86.3

All of the strains of meningococci so far studied, except 12, were agglutinated by one or the other of the type serums. Of the 36 strains obtained from cases of meningitis 22 were from sporadic cases occurring in Chicago. Nineteen of these, or 86.3%, were found to belong to Group 2. Furthermore in two instances in which the contacts were examined, carriers were found which harbored organisms of the same biologic type. On the other hand, the meningococci isolated from cases of meningitis and chronic carriers in the military camp were found to belong to Group 1 in most instances. Of the case strains 85.7% and of the carrier strains 83.3% were classified in Group 1. The interesting fact emerges from this summary that the prevailing types of meningococcus causing meningitis in the two localities differed in their biologic reactions. But the organisms isolated from cases of meningitis and carriers in the same locality were similar in biologic reaction in the majority of instances.

In the agglutination experiments marked differences were observed in the agglutinability and agglutinogenic properties of certain strains of meningococci. Of the 150 strains examined 121 yielded specific group reactions and were classifiable in one or the other of the 2 groups. Twenty-nine strains were irregular in their biologic reactions. Seventeen of these atypical strains were agglutinated by representative serums of both groups. In all instances, however, the dilution of serum in which agglutination of these organisms occurred was much higher with one group than the other. These meningococci were classified in the group represented by the serum with the highest agglutinin content for the particular strain. To gain further information of the biologic reactions of these irregular strains monovalent serum was prepared from 4 of the 17 irregular organisms. These monovalent serums, however, yielded specific reactions with meningococci of one or the other of the two main groups as indicated by the results of the original agglutination tests. The remaining 12 of the atypical strains were not agglutinated by the group monovalent serums or the polyvalent serums. Eleven of these inagglutinable strains were obtained from carriers and one from the cerebrospinal fluid of a patient with meningitis. All of these organisms revealed the typical cultural and morphologic characters of the meningococcus. Monovalent serums prepared from these strains agglutinated in a specific way meningococci belonging to one or the other of the large biologic groups. In this manner it has been possible to classify all the inagglutinable strains so far encountered (See Diagram).

DIAGRAM SHOWING GROUPING OF MENINGOCOCCI



The atypical subgroups includes strains with variable agglutinative reactions and tendency to cross agglutination.

It is interesting to note that the biologic reactions of the carrier strains of meningococci are atypical in more instances than those of the case strains. Furthermore, differences in agglutinability were observed among meningococci belonging to the same group.

Clinically certain Group 2 (parameningococcus) infections have not reacted to treatment with polyvalent serum of high potency. These cases run a chronic course and terminate fatally after a few weeks. It seems probable that a monovalent Group 2 serum might be efficacious in such cases.

Meningococci with biologic characters similar to those of the atypical strains met with in this work have been the subject of much discussion. Some investigators have been content to call them intermediates without attempting to classify them. Gordon and his associates have classified these variants into two biologic groups (Groups 3 and 4). The importance of these debatable biologic groups, however, seems more apparent than real for they are closely related to the two main groups.

The results of the immunologic observations described herein suggest that the meningococcus can be divided into two large groups on the basis of biologic reactions which correspond to the meningococcus and parameningococcus as described by Dopter and others. The members of these large groups, however, vary in their agglutinability and agglutinogenic properties within wide limits, and include typical, irregular and inagglutinable organisms.

SUMMARY

During the summer and fall of 1917 an extensive bacteriologic study was made of epidemic meningitis in one of the large military camps. The meningococcus carriers in the infected organizations were identified and isolated. For the cultures plain blood agar was found to be satisfactory and the material for culture was obtained from the nasopharynx by means of a simple uncovered wire swab. It was found that 3-6% of the men examined were meningococcus carriers. The majority of these carriers, however, were of the temporary type; only 1.2% of the total number of suspects examined proved to be chronic carriers. Chronic meningococcus carriers, as distinguished from the temporary type, often harbor great numbers of meningococci in the secretions of the nose and throat. The number of carriers was found to be high among those coming in contact with meningitis cases.

In a study of the biologic reactions of 150 strains of meningococci from different sources two large biologic groups were differentiated by means of macroscopic agglutination tests using monovalent serums. The agglutination reactions were in most instances definite and specific but a number of atypical and inagglutinable strains were met with in each group. The atypical strains however did not differ enough from the other members of the group to warrant different classification as

determined by agglutination. The classification of the inagglutinable strains was accomplished by means of agglutination with monovalent serums prepared from these strains; these serums yielded specific reactions with organisms of one or the other main type.

The biologic type of meningococcus predominant in the camp epidemic was identical with the type prevailing among the chronic carriers, but different from the type of meningococcus causing the majority of the sporadic cases of cerebrospinal fever in Chicago. Also the type of meningococcus found in the cases and in the corresponding immediate contacts was in every instance the same. These facts suggest that there is a close relationship between cases of epidemic meningitis and meningococcus carriers.